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<110> Eck, Jurgen
Schmidt, Arno
Zinke, Holger

<120> Recombinant Fusion Proteins Based on Ribosome-Inactivating Proteins of the Mistletoe Viscum album

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<141> 1999-07-02

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gagcgatacg ccggacatag ggaccagatc cctctcggta tagaccaact cattcaatcc 420

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20 25 30

Ser Phe Ser Asn Glu Ile Pro Leu Leu Arg Gln Ser Thr Ile Pro Val
35 40 45

Ser Asp Ala Gln Arg Phe Val Leu Val Glu Leu Thr Asn Gln Gly Gly
50 55 60

Asp Ser Ile Thr Ala Ala Ile Asp Val Thr Asn Leu Tyr Val Val Ala
65 70 75 80

Tyr Gln Ala Gly Asp Gln Ser Tyr Phe Leu Arg Asp Ala Pro Arg Gly
85 90 95

Ala Glu Thr His Leu Phe Thr Gly Thr Thr Arg Ser Ser Leu Pro Phe
100 105 110

Asn Gly Ser Tyr Pro Asp Leu Glu Arg Tyr Ala Gly His Arg Asp Gln
115 120 125

Ile Pro Leu Gly Ile Asp Gln Leu Ile Gln Ser Val Thr Ala Leu Arg
130 135 140

Phe Pro Gly Gly Ser Thr Arg Thr Gln Ala Arg Ser Ile Leu Ile Leu
145 150 155 160

Ile Gln Met Ile Ser Glu Ala Ala Arg Phe Asn Pro Ile Leu Trp Arg
165 170 175

Ala Arg Gln Tyr Ile Asn Ser Gly Ala Ser Phe Leu Pro Asp Val Tyr
180 185 190

Met Leu Glu Leu Glu Thr Ser Trp Gly Gln Gln Ser Thr Gln Val Gln
195 200 205

His Ser Thr Asp Gly Val Phe Asn Asn Pro Ile Arg Leu Ala Ile Pro
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245 250

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cagttgtggc cctccaagtc caacaatgat ccgaatcagt tgtggacgat caaaagggat 180

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aatgggacca tcatcaatcc aagatccaat ctggttttgg cagcatcatc tggaatcaaa     360

ggcactacgc ttacggtgca aacactggat tacacgttgg gacagggctg gcttgccggt     420

aatgataccg ccccacgcga ggtgaccata tatgggttca gggacctttg catggaatca     480

aatggaggga gtgtgtgggt ggagacgtgc gtgagtagcc aaaagaacca aagatgggct     540

ttgtacgggg atggttctat acgccccaaa caaaaccaag accaatgcct cacctgtggg     600

agagactccg tttcaacagt aatcaatata gttagctgca gcgctggatc gtctgggcag     660

cgatgggtgt ttaccaatga aggggccatt ttgaatttaa agaatgggtt ggccatggat     720

gtggcgcaag caaatccaaa gctccgccga ataatcatct atcctgccac aggaaaacca     780

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Arg Asn Gly Met Cys Val Asp Val Arg Asp Asp Asp Phe Arg Asp Gly
            20                  25                  30

Asn Gln Ile Gln Leu Trp Pro Ser Lys Ser Asn Asn Asp Pro Asn Gln
        35                  40                  45

Leu Trp Thr Ile Lys Arg Asp Gly Thr Ile Arg Ser Asn Gly Ser Cys
    50                  55                  60

Leu Thr Thr Tyr Gly Tyr Thr Ala Gly Val Tyr Val Met Ile Phe Asp
65                  70                  75                  80

Cys Asn Thr Ala Val Arg Glu Ala Thr Leu Trp Gln Ile Trp Gly Asn
                85                  90                  95

Gly Thr Ile Ile Asn Pro Arg Ser Asn Leu Val Leu Ala Ala Ser Ser
            100                 105                 110

Gly Ile Lys Gly Thr Thr Leu Thr Val Gln Thr Leu Asp Tyr Thr Leu
        115                 120                 125

Gly Gln Gly Trp Leu Ala Gly Asn Asp Thr Ala Pro Arg Glu Val Thr
    130                 135                 140

Ile Tyr Gly Phe Arg Asp Leu Cys Met Glu Ser Asn Gly Gly Ser Val
145                 150                 155                 160

Trp Val Glu Thr Cys Val Ser Ser Gln Lys Asn Gln Arg Trp Ala Leu
                165                 170                 175

Tyr Gly Asp Gly Ser Ile Arg Pro Lys Gln Asn Gln Asp Gln Cys Leu
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180 185 190

Thr Cys Gly Arg Asp Ser Val Ser Thr Val Ile Asn Ile Val Ser Cys
195 200 205

Ser Ala Gly Ser Ser Gly Gln Arg Trp Val Phe Thr Asn Glu Gly Ala
210 215 220

Ile Leu Asn Leu Lys Asn Gly Leu Ala Met Asp Val Ala Gln Ala Asn
225 230 235 240

Pro Lys Leu Arg Arg Ile Ile Ile Tyr Pro Ala Thr Gly Lys Pro Asn
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Gln Met Trp Leu Pro Val Pro Gly Gly Tyr His
260 265

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gatgttacat gt 72

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1 5 10 15

Ala

<210> 7
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atcacgcttc tccgagatta tgtctcaagc ggaagctttt ccaatgagat accactcttg 120

cgtcagtcta cgatccccgt ctccgatgcg caaagatttg tcttggtgga gctcaccaac 180

caggggggag actcgatcac ggccgccatc gacgttacca atctgtacgt cgtggcttac 240

caagcaggcg accaatccta ctttttgcgc gacgcaccac gcggcgcgga aacgcatctc 300

ttcaccggca ccacccgatc ctctctccca ttcaacggaa gctaccctga tctggagcga 360

tacgccggac atagggacca gatccctctc ggtatagacc aactcattca atccgtcacg 420

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aacagtgggg cgtcatttct gccagacgtg tacatgctgg agctggagac gagttggggc     600

caacaatcca cgcaagtcca gcattcaacc gatggcgttt ttaataaccc aattcggttg     660

gctatacccc ccggtaactt cgtgacgttg accaatgttc gcgacgtgat cgccagcttg     720

gcgatcatgt tgtttgtatg cggagagcgg ccatct                               756


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Tyr Phe Arg Phe Ile Thr Leu Leu Arg Asp Tyr Val Ser Ser Gly Ser
            20                  25                  30

Phe Ser Asn Glu Ile Pro Leu Leu Arg Gln Ser Thr Ile Pro Val Ser
        35                  40                  45

Asp Ala Gln Arg Phe Val Leu Val Glu Leu Thr Asn Gln Gly Gly Asp
    50                  55                  60

Ser Ile Thr Ala Ala Ile Asp Val Thr Asn Leu Tyr Val Val Ala Tyr
65                  70                  75                  80

Gln Ala Gly Asp Gln Ser Tyr Phe Leu Arg Asp Ala Pro Arg Gly Ala
                85                  90                  95

Glu Thr His Leu Phe Thr Gly Thr Thr Arg Ser Ser Leu Pro Phe Asn
            100                 105                 110

Gly Ser Tyr Pro Asp Leu Glu Arg Tyr Ala Gly His Arg Asp Gln Ile
        115                 120                 125

Pro Leu Gly Ile Asp Gln Leu Ile Gln Ser Val Thr Ala Leu Arg Phe
    130                 135                 140

Pro Gly Gly Ser Thr Arg Thr Gln Ala Arg Ser Ile Leu Ile Leu Ile
145                 150                 155                 160

Gln Met Ile Ser Glu Ala Ala Arg Phe Asn Pro Ile Leu Trp Arg Ala
                165                 170                 175

Arg Gln Tyr Ile Asn Ser Gly Ala Ser Phe Leu Pro Asp Val Tyr Met
            180                 185                 190

Leu Glu Leu Glu Thr Ser Trp Gly Gln Gln Ser Thr Gln Val Gln His
        195                 200                 205

Ser Thr Asp Gly Val Phe Asn Asn Pro Ile Arg Leu Ala Ile Pro Pro
    210                 215                 220

Gly Asn Phe Val Thr Leu Thr Asn Val Arg Asp Val Ile Ala Ser Leu
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Ala Ile Met Leu Phe Val Cys Gly Glu Arg Pro Ser
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tgcgtggacg tccgagatga cgatttccgc gatggaaatc agatacagtt gtggccctcc     120

aagtccaaca atgatccgaa tcagttgtgg acgatcaaaa gggatggaac cattcgatcc     180

aatggcagct gcttgaccac gtatggctat actgctggcg tctatgtgat gatcttcgac     240

tgtaatactg ctgtgcggga ggccactctt tggcagatat ggggcaatgg gaccatcatc     300

aatccaagat ccaatctggt tttggcagca tcatctggaa tcaaaggcac tacgcttacg     360

gtgcaaacac tggattacac gttgggacag ggctggcttg ccggtaatga taccgcccca     420

cgcgaggtga ccatatatgg gttcagggac ctttgcatgg aatcaaatgg agggagtgtg     480

tgggtggaga cgtgcgtgag tagccaaaag aaccaaagat gggctttgta cggggatggt     540

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acagtaatca atatagttag ctgcagcgct ggatcgtctg ggcagcgatg ggtgtttacc     660

aatgaagggg ccattttgaa tttaaagaat gggttggcca tggatgtggc gcaagcaaat     720

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cccgtgcca                                                             789


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Arg Asn Gly Met Cys Val Asp Val Arg Asp Asp Asp Phe Arg Asp Gly
            20                  25                  30

Asn Gln Ile Gln Leu Trp Pro Ser Lys Ser Asn Asn Asp Pro Asn Gln
        35                  40                  45

Leu Trp Thr Ile Lys Arg Asp Gly Thr Ile Arg Ser Asn Gly Ser Cys
    50                  55                  60

Leu Thr Thr Tyr Gly Tyr Thr Ala Gly Val Tyr Val Met Ile Phe Asp
65                  70                  75                  80
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Cys Asn Thr Ala Val Arg Glu Ala Thr Leu Trp Gln Ile Trp Gly Asn
85 90 95

Gly Thr Ile Ile Asn Pro Arg Ser Asn Leu Val Leu Ala Ala Ser Ser
100 105 110

Gly Ile Lys Gly Thr Thr Leu Thr Val Gln Thr Leu Asp Tyr Thr Leu
115 120 125

Gly Gln Gly Trp Leu Ala Gly Asn Asp Thr Ala Pro Arg Glu Val Thr
130 135 140

Ile Tyr Gly Phe Arg Asp Leu Cys Met Glu Ser Asn Gly Gly Ser Val
145 150 155 160

Trp Val Glu Thr Cys Val Ser Ser Gln Lys Asn Gln Arg Trp Ala Leu
165 170 175

Tyr Gly Asp Gly Ser Ile Arg Pro Lys Gln Asn Gln Asp Gln Cys Leu
180 185 190

Thr Cys Gly Arg Asp Ser Val Ser Thr Val Ile Asn Ile Val Ser Cys
195 200 205

Ser Ala Gly Ser Ser Gly Gln Arg Trp Val Phe Thr Asn Glu Gly Ala
210 215 220

Ile Leu Asn Leu Lys Asn Gly Leu Ala Met Asp Val Ala Gln Ala Asn
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Pro Lys Leu Arg Arg Ile Ile Ile Tyr Pro Ala Thr Gly Lys Pro Asn
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Gln Met Trp Leu Pro Val Pro
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<210> 11
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<212> DNA
<213> Viscum album

<400> 11
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<210> 12
<211> 16
<212> PRT
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<400> 12

Ser Ser Glu Val Arg Tyr Trp Pro Leu Val Ile Arg Pro Val Ile Ala
1 5 10 15

<210> 13
<211> 94
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<220>
<223> Synthetic gene encoding amino acids 53-78 of human P2 protein

<400> 13
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ggtcaggaat tcgaagaaac caccgctgac aact   94

<210> 14
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<212> PRT
<213> Artificial Sequence

<220>
<223> Amino acids 53-78 of human P2 protein

<400> 14

Arg Thr Glu Ser Thr Phe Lys Asn Thr Glu Ile Ser Phe Lys Leu Gly
1   5   10   15

Gln Glu Phe Glu Glu Thr Thr Ala Asp Asn
            20   25

<210> 15
<211> 75
<212> DNA
<213> Artificial Sequence

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<223> Figure 20: Synthetic linker cassette for providing modularity at
       the 3' end of rMLB delta 1 alpha 1 beta

<400> 15
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gtaaggatcc ctcga   75

<210> 16
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Amino acid sequence encoded by portion of SEQ ID NO: 15

<400> 16

Thr Gly Lys Pro Asn Gln Met Trp Leu Pro Val Pro
1   5   10

<210> 17
<211> 82
<212> DNA
<213> Artificial Sequence

<220>
<223> Fig. 21: Synthetic linker cassette for providing modularity at

the 3'end of rMLB Delta 1 alpha 1 beta 2 gamma with affinity module ("His-Tag").

<400> 17
ccggtaaacc gaaccagatg tggctgccgg taccgggtgg tggatatcat caccaccatc 60

accactagta actcctcgga tc 82

<210> 18
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<220>
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<400> 18

Gly Lys Pro Asn Gln Met Trp Leu Pro Val Pro Gly Gly Gly Tyr His
1 5 10 15

His His His His His
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<210> 19
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<210> 20
<211> 27
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<223> Fig. 22: Mutagenic oligonucleotides for inactivating carbohydrate binding sites in rMLB. - 1alpha2 (W38A).

<400> 20
cagatacagt tggcgccctc caagtcc 27

<210> 21
<211> 61
<212> DNA
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<223> Fig. 22:Mutagenic oligonucleotides for inactivating carbohydrate binding sites in rMLB. - 1beta (Y68S,Y70S, Y75S, F79S).

<400> 21
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c 61

<210> 22
<211> 26
<212> DNA
<213> Artificial Sequence

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<223> Fig. 22: Mutagenic oligonucleotides for inactivating carbohydrate binding sites in rMLB. - 1beta1 (D235A).

<400> 22
gggttggcca tggctgtggc gcaagc 26

<210> 23
<211> 26
<212> DNA
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<223> Fig. 22: Mutagenic oligonucleotides for inactivating carbohydrate binding sites in rMLB. - 2gamma2 (Y249A). -

<400> 23
cgaataatca tcgctcctgc cacagg 26

<210> 24
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> Fig. 22: Mutagenic oligonucleotides for inactivating carbohydrate binding sites in rMLB. - pT7 EcoRV to SspI.

<400> 24
cttccttttt caatattatt gaagcattta tcagg 35

<210> 25
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> Fig. 22: Mutagenic oligonucleotides for inactivating carbohydrate binding sites in rMLB. - pT7 SspI to EcoRV. -

<400> 25
cttccttttt cgatatcatt gaagcattta tcagg 35

<210> 26
<211> 40
<212> DNA
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<220>
<223> Fig. 23: Mutagenic oligonucleotides for constructing modular ITF

gene cassettes. - pT7 Delta NdeI to StuI.

<400> 26
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<210> 27
<211> 33
<212> DNA
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<223> Fig. 23: Mutagenic oligonucleotides for constructing modular ITF gene cassettes. - rMLB silent NheI.

<400> 27
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<210> 28
<211> 32
<212> DNA
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<220>
<223> Fig. 23: Mutagenic oligonucleotides for constructing modular ITF gene cassettes. - rMLA Delta AgeI.

<400> 28
cccaccagac caccggcgaa gaatatttcc gg 32

<210> 29
<211> 40
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<220>
<223> Fig. 23: Mutagenic oligonucleotides for constructing modular ITF gene cassettes.

<400> 29
gtttgtatgc ggagagcgtc cctcgagctc tgaggtgcgc 40

<210> 30
<211> 43
<212> DNA
<213> Artificial Sequence

<220>
<223> Fig. 23: Mutagenic oligonucleotides for constructing modular ITF gene cassettes. - rMLB Delta EcoNI to AgeI.

<400> 30
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<210> 31
<211> 11
<212> DNA
<213> Artificial Sequence

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<223> Flanking region of the ProML gene cassette in expression vector pT7ProML

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tacatatgta c 11

<210> 32
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<220>
<223> Flanking region of the ProML gene cassette in expression vector pT7ProML

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<210> 33
<211> 9
<212> DNA
<213> Artificial Sequence

<220>
<223> Flanking region of the IML gene cassette in expression vector PIMML-02-P

<400> 33
caggcctac 9

<210> 34
<211> 34
<212> DNA
<213> Artificial Sequence

<220>
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<211> 4
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<213> Artificial Sequence

<220>
<223> Modulator module peptide

<400> 35

Lys Asp Glu Leu
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<210> 36
<211> 4

<212> PRT
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<220>
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<400> 36

His Asp Glu Leu
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<210> 37
<211> 16
<212> PRT
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<220>
<223> Portion of the ML propeptide

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1   5   10   15

<210> 38
<211> 13
<212> PRT
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<220>
<223> A degredation product of mylein basic protein

<400> 38

Val His Phe Phe Lys Asn Ile Val Thr Pro Arg Thr Pro
1   5   10

<210> 39
<211> 34
<212> DNA
<213> Artificial Sequence

<220>
<223> Figure 1.a: bFGF specific primer for PCR amplification of the bFGF gene (5' to 3')

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<210> 40
<211> 32
<212> DNA
<213> Artificial Sequence

<220>
<223> Figure 1.a: bFGF specific primer for PCR amplification of the bFGF gene (3' to 5')

<400> 40

gaaggttaca gacgattctc ggtatactta at 32

<210> 41
<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<223> Figure 1.b: DNA sequence encoding C-terminal processing sequence of bFGF

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tctgctaaga gccat 15

<210> 42
<211> 5
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<213> Artificial Sequence

<220>
<223> Figure 1.b: Amino acid sequence of C-terminal processing sequence of bFGF

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1 5

<210> 43
<211> 756
<212> DNA
<213> Viscum album

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cgtcagtcta cgatccccgt ctccgatgcg caaagatttg tcttggtgga gctcaccaac 180

caggggggag actcgatcac ggccgccatc gacgttacca atctgtacgt cgtggcttac 240

caagcaggcg accaatccta ctttttgcgc gacgcaccac gcggcgcgga aacgcatctc 300

ttcaccggca ccacccgatc ctctctccca ttcaacggaa gctaccctga tctggagcga 360

tacgccggac atagggacca gatccctctc ggtatagacc aactcattca atccgtcacg 420

gcgcttcgtt ttccgggcgg cagcacgcgt acccaagctc gttcgatttt aatcctcatt 480

cagatgatct ccgaggccgc cagattcaat cccatcttat ggagggctcg ccaatacatt 540

aacagtgggg cgtcatttct gccagacgtg tacatgctgg agctggagac gagttggggc 600

caacaatcca cgcaagtcca gcattcaacc gatggcgttt ttaataaccc aattcggttg 660

gctatacccc ccggtaactt cgtgacgttg accaatgttc gcgacgtgat cgccagcttg 720

gcgatcatgt tgtttgtatg cggagagcgg ccatct 756

<210> 44
<211> 252
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20 25 30

Phe Ser Asn Glu Ile Pro Leu Leu Arg Gln Ser Thr Ile Pro Val Ser
35 40 45

Asp Ala Gln Arg Phe Val Leu Val Glu Leu Thr Asn Gln Gly Gly Asp
50 55 60

Ser Ile Thr Ala Ala Ile Asp Val Thr Asn Leu Tyr Val Val Ala Tyr
65 70 75 80

Gln Ala Gly Asp Gln Ser Tyr Phe Leu Arg Asp Ala Pro Arg Gly Ala
85 90 95

Glu Thr His Leu Phe Thr Gly Thr Thr Arg Ser Ser Leu Pro Phe Asn
100 105 110

Gly Ser Tyr Pro Asp Leu Glu Arg Tyr Ala Gly His Arg Asp Gln Ile
115 120 125

Pro Leu Gly Ile Asp Gln Leu Ile Gln Ser Val Thr Ala Leu Arg Phe
130 135 140

Pro Gly Gly Ser Thr Arg Thr Gln Ala Arg Ser Ile Leu Ile Leu Ile
145 150 155 160

Gln Met Ile Ser Glu Ala Ala Arg Phe Asn Pro Ile Leu Trp Arg Ala
165 170 175

Arg Gln Tyr Ile Asn Ser Gly Ala Ser Phe Leu Pro Asp Val Tyr Met
180 185 190

Leu Glu Leu Glu Thr Ser Trp Gly Gln Gln Ser Thr Gln Val Gln His
195 200 205

Ser Thr Asp Gly Val Phe Asn Asn Pro Ile Arg Leu Ala Ile Pro Pro
210 215 220

Gly Asn Phe Val Thr Leu Thr Asn Val Arg Asp Val Ile Ala Ser Leu
225 230 235 240

Ala Ile Met Leu Phe Val Cys Gly Glu Arg Pro Ser
245 250

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Arg Asn Gly Met Cys Val Asp Val Arg Asp Asp Asp Phe Arg Asp Gly
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Asn Gln Ile Gln Leu Trp Pro Ser Lys Ser Asn Asn Asp Pro Asn Gln
35 40 45

Leu Trp Thr Ile Lys Arg Asp Gly Thr Ile Arg Ser Asn Gly Ser Cys
    50   55   60

Leu Thr Thr Tyr Gly Tyr Thr Ala Gly Val Tyr Val Met Ile Phe Asp
65   70   75   80

Cys Asn Thr Ala Val Arg Glu Ala Thr Leu Trp Gln Ile Trp Gly Asn
                85   90   95

Gly Thr Ile Ile Asn Pro Arg Ser Asn Leu Val Leu Ala Ala Ser Ser
            100   105   110

Gly Ile Lys Gly Thr Thr Leu Thr Val Gln Thr Leu Asp Tyr Thr Leu
        115   120   125

Gly Gln Gly Trp Leu Ala Gly Asn Asp Thr Ala Pro Arg Glu Val Thr
    130   135   140

Ile Tyr Gly Phe Arg Asp Leu Cys Met Glu Ser Asn Gly Gly Ser Val
145   150   155   160

Trp Val Glu Thr Cys Val Ser Ser Gln Lys Asn Gln Arg Trp Ala Leu
                165   170   175

Tyr Gly Asp Gly Ser Ile Arg Pro Lys Gln Asn Gln Asp Gln Cys Leu
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Thr Cys Gly Arg Asp Ser Val Ser Thr Val Ile Asn Ile Val Ser Cys
        195   200   205

Ser Ala Gly Ser Ser Gly Gln Arg Trp Val Phe Thr Asn Glu Gly Ala
    210   215   220

Ile Leu Asn Leu Lys Asn Gly Leu Ala Met Asp Val Ala Gln Ala Asn
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Pro Lys Leu Arg Arg Ile Ile Ile Tyr Pro Ala Thr Gly Lys Pro Asn
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Gln Met Trp Leu Pro Val Pro
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